

AMENDMENTS TO THE CLAIMS

Please amend claims 1, 5-9, 12-15, 18-20, 22, 26, 32-36, and 39-41, cancel claims 3, 4, and 27-30,, and add new claim 42 as set forth below, without prejudice or disclaimer. Withdraw claim 31 and 37, without prejudice or disclaimer.

The current listing of claims replaces all prior listings.

1. (Currently Amended) A method of treating critical limb ischemia (CLI) comprising administering intramuscularly to a patient in need of treatment an effective amount of a polynucleotide encoding a mutant mammalian endothelial nitric oxide synthase (eNOS) polypeptide, wherein the eNOS polypeptide comprises as least one mutation at a position corresponding to an amino acid residue in a calmodulin-binding domain that is phosphorylated in wild-type eNOS in mammalian cells, said calmodulin-binding domain corresponding to amino acid residues 478-522 of SEQ ID NO:1.
2. (Original) The method according to Claim 1, wherein said eNOS polypeptide is a human eNOS polypeptide.
3. (Canceled)
4. (Canceled)
5. (Currently Amended) The method according to Claim 1[[4]], wherein said eNOS polypeptide comprises a mutation at a position corresponding to amino acid residue 495 of SEQ ID NO: 1.

6. (Currently Amended) The method according to Claim 1[[4]], wherein said eNOS polypeptide further comprises a mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1.

7. (Currently Amended) The method according to Claim 1[[4]], wherein said eNOS polypeptide comprises a first mutation at a position corresponding to amino acid 495 and a second mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1.

8. (Currently Amended) The method according to Claim 1[[4]], wherein said eNOS polypeptide comprises a first mutation at a position corresponding to amino acid 495, a second mutation at a position corresponding to amino acid 1177, and a third mutation at a position corresponding to amino acid 2 of SEQ ID NO: 1.

9. (Currently Amended) The method according to Claim [[6]] 5, 7, or 8, wherein said mutation at a position corresponding to amino acid residue 495 is an amino acid substitution to Ala, Val, Leu, or Ile.

10. (Original) The method according to Claim 6, 7, or 8, wherein said mutation at a position corresponding to amino acid residue 1177 is an amino acid substitution to Asp.

11. (Original) The method according to Claim 8, wherein said mutation at a position corresponding to amino acid residue 2 is an amino acid substitution to Ala.

12. (Currently Amended) The method according to Claim 1[[4]], wherein the phosphorylation of said eNOS polypeptide is ~~increased or decreased~~, as compared to a ~~reference~~ wild-type eNOS polypeptide.

13. (Currently Amended) The method according to Claim 1[[4]], wherein said eNOS polypeptide has an increased binding affinity for calmodulin, as compared to a ~~reference~~ wild-type eNOS polypeptide.
14. (Currently Amended) The method according to Claim 1[[4]], wherein Ca⁺⁺ dependence is decreased in Ca⁺⁺-calmodulin mediated stimulation of said eNOS polypeptide as compared to a ~~reference~~ wild-type eNOS polypeptide.
15. (Currently Amended) The method according to Claim 1[[4]], wherein said eNOS polypeptide has increased eNOS activity, as compared to a ~~reference~~ wild-type eNOS polypeptide.
16. (Original) The method according to Claim 15, wherein said activity is the generation of NO.
17. (Original) The method according to Claim 15, wherein said activity is reductase activity.
18. (Currently Amended) The method according to Claim 12, 13, 14, 15, 16, or 17, wherein the amino acid sequence of said ~~reference~~ wild-type polypeptide is, ~~or is derived from,~~ the amino acid sequence of a human eNOS.
19. (Currently Amended) The method according to Claim 18, wherein the amino acid sequence of said ~~reference~~ wild-type polypeptide is, ~~or is derived from,~~ SEQ ID NO: 1.
20. (Currently Amended) The method according to Claim 1[[4]], wherein the amino acid sequence of said eNOS polypeptide comprises mutations at amino acid residues corresponding to

threonine 495 and serine 1177 is substantially homologous to the amino acid sequence of a human eNOS comprising the amino acid sequence of SEQ ID NO:1.

21. (Original) The method according to Claim 20, wherein the amino acid sequence of said eNOS polypeptide has a 95-99 % sequence identity to the amino acid sequence of SEQ ID NO:

1.

22. (Currently Amended) The method according to Claim 1 or 12[[4]], wherein said polynucleotide is a recombinant vector comprising a nucleic acid sequence encoding said eNOS polypeptide and said sequence is operably linked to at least one regulatory sequence such that said polypeptide is expressed in cells.

23. (Original) The method according to Claim 22, wherein said nucleic acid sequence is operably linked to a promoter.

24. (Original) The method according to Claim 23, wherein said recombinant vector is a viral vector.

25. (Original) The method according to Claim 24, wherein said viral vector is an adenoviral vector.

26. (Currently Amended) The method according to Claim 1 or 12[[4]], wherein said treating comprises modulating eNOS activity in cells of said patient.

27-30. (Canceled)

31. (Withdrawn) The method according to Claim 1 or 4, wherein said administering comprises introducing said polynucleotide to cells of said patient ex vivo.

32. (Currently Amended) The method according to Claim 1 or 12[[4]], wherein said administering comprises delivery of said polynucleotide to a diseased tissue of said patient.

33. (Currently Amended) The method according to Claim 1 or 12[[4]], wherein said administering comprises delivery of said polynucleotide to the peripheral vascular system of said patient.

34. (Currently Amended) The method according to Claim 33, wherein said delivery is by ~~intramuscular injection or intraarterial injection~~ to a limb muscle of said patient.

35. (Currently Amended) A method of treating an angiogenesis-dependent disorder comprising administering intramuscularly to a patient in need of treatment an effective amount of a polynucleotide encoding [[an]] a mutant mammalian endothelial nitric oxide synthase (eNOS) polypeptide, wherein said eNOS polypeptide comprises at least one mutation at a position corresponding to an amino acid residue in a calmodulin-binding domain mammalian eNOS that is phosphorylated in wild-type eNOS in mammalian cells, said calmodulin-binding domain corresponding to amino acid residues 478-522 of SEQ ID NO:1.

36. (Currently Amended) A method of ameliorating microvascular dysfunction comprising administering intramuscularly to a patient in need of treatment an effective amount of a polynucleotide encoding [[an]] a mutant mammalian endothelial nitric oxide synthase (eNOS) polypeptide, wherein said eNOS polypeptide comprises at least one mutation at a position corresponding to an amino acid residue in a calmodulin-binding domain mammalian eNOS that is

phosphorylated in wild-type eNOS in mammalian cells, said calmodulin-binding domain corresponding to amino acid residues 478-522 of SEQ ID NO:1.

37. (Withdrawn) A method of treating critical limb ischemia (CLI) comprising administering to a patient in need of treatment an effective amount of an eNOS polypeptide, wherein said eNOS polypeptide comprises at least one mutation at a position corresponding to an amino acid residue in a mammalian eNOS that is phosphorylated in mammalian cells.

38. (Currently Amended) The method according to Claim 35[[,]] or 36, [[or 37,]] wherein said eNOS polypeptide comprises a mutation at a position corresponding to amino acid residue 495 of SEQ ID NO: 1, and said mutation is an amino acid substitution to Ala, Val, Leu, or Ile.

39. (Currently Amended) The method according to Claim 35[[,]] or 36, [[or 37,]] wherein said eNOS polypeptide further comprises a mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1, and said mutation is an amino acid substitution to Asp.

40. (Currently Amended) The method according to Claim [[1,]] 35[[,]] or 36, [[or 37,]] wherein said eNOS polypeptide comprises:

- i) a first mutation at a position corresponding to amino acid 495 of SEQ ID NO:1, and said first mutation is an amino acid substitution to Ala, Val, Leu, or Ile; and
- ii) a second mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1, and said second mutation is an amino acid substitution to Asp.

41. (Currently Amended) The method according to Claim [[1,]] 35[[,]] or 36, [[or 37,]] wherein said eNOS polypeptide comprises:

- i) a first mutation at a position corresponding to amino acid 495 of SEQ ID NO:1, and said first mutation is an amino acid substitution to Ala, Val, Leu, or Ile;

In re Application of:

Dole et al.

Application No.: 10/642,255

Filing Date: August 15, 2003

Page 8

PATENT

Attorney Docket No. CARD1110-1

ii) a second mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1, and said second mutation is an amino acid substitution to Asp; and

iii) a third mutation at a position corresponding to amino acid 2 of SEQ ID NO: 1, and said second mutation is an amino acid substitution to Ala.

42. (New) The method according to any of Claims 9, 38, 40, or 41, wherein said mutation at a position corresponding to amino acid residue 495 is an amino acid substitution to Val.